STRUCTURAL VARIATION IN *SBDS* GENE, WITH LOSS OF EXON 3, IN TWO NEW SHWACHMAN-DIAMOND PATIENTS

Minelli A[1], Nacci L[1], Valli R[2], Pietrocola G[3], Ramenghi U[4], Locatelli F[5], Brescia L[5], Nicolis E[6], Cipolli M[7], Danesino C[1].

[1]Medical Genetics Unit, Department of Molecular Medicine, University of Pavia, Pavia, Italy
[2]Human and Medical Genetics, Department of Clinical and Experimental Medicine, University of Insubria, Varese, Italy
[3] Unit of Biochemistry, Department of Molecular Medicine, University of Pavia, Pavia, Italy
[4] Pediatric Hematology, Department of Public Health and Pediatric Sciences, University of Torino, Torino, Italy
[5]Department of Pediatric Hematology and Oncology, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy
[6]Laboratory of Transfusion Medicine, University Hospital of Verona, Verona, Italy
[7]Cystic Fibrosis Centre, Azienda Ospedaliera Universitaria, Verona, Italy

Shwachman-Diamond Syndrome (SDS) is an autosomal recessive disease (#260400) characterized by pancreatic exocrine insufficiency, haematological abnormalities (neutropenia and anaemia), bone marrow failure (with increased risk for acute myeloid leukaemia (AML) or myelodysplasia (MDS) and skeletal alterations.

As suggested by international guidelines[1], the SDS clinical diagnosis is confirmed by detection of bi-allelic pathogenic variants in *SBDS* gene, localized at 7q11.21[2,3].

Targeted mutation analysis of exon 2 by PCR_RFLP discloses in at least 90% of SDS patients one of the three common mutations, (c.185_184TA>CT, c.258+2T>C and the combination of c.[183_184TA>CT:258+2T>C] on one allele). Two of the common mutations are observed concomitantly in approximately 62% of SDS patients[3].

In our laboratory, starting from 2003, we performed *SBDS* molecular analysis confirming the clinical diagnosis in 115 patients, and all of them harboured at least one of the classical mutations in exon 2.

We recently found two families, each of them with two affected children (UPN 42, UPN 43 and UPN 61, UPN 76), carrying only the [c.258+2T>C] mutation; no other DNA changes was found after complete sequencing analysis of all five exons and their flanking intronic regions of *SBDS*. In both families, the clinical picture of the patients completely fits the diagnostic criteria.

Western-blot analysis using SBDS antibody did not demonstrate any SBDS protein in these patients, suggesting the presence of a more complex anomaly on the second allele (Fig. 1).

We performed a deletion/duplication analysis by long-range gDNA PCR with the specific primers designed as Boocock et al.[2], but matching them differently to obtain longer amplicons containing more exons. When using the following primers [forward for exon 2] and [reverse for exon 4], that amplify the region from exon 2 to exon 4, we observed in both index cases the expected band of 3756 bp and the presence of a second smaller band of approximately 3000 bp (Fig. 2).

We then extracted the DNA from the smaller band and used it as template in a new long-range PCR experiment, with the same primers as before. Direct sequencing of PCR product demonstrated a deletion of exon 3; breakpoints at 5' and 3' are located, respectively, in intron 2 and in intron 3.

The coordinates of the deleted region in GRCh37/hg19 assembly are chr7: g.66457801-66458666, with a deletion size of 866 bp. According to nomenclature for sequence variations[4], we describe the mutation as [c.258+533_459+403del]. The presence of the mutation was confirmed in the father of UPN 42/43 and in the mother of UPN 61/76.

The two families we studied are unrelated, the family names of the two parents carrying the deletion of exon 3 are uncommon in Italy, and have their higher incidence in different Italian areas, far apart from each other, as Sicily and Lazio; available evidence suggest that the mutations arose independently in the two families.

In the 92 index cases known to the Italian registry, mutations other than the common ones are 14, only one of them [c.187G>T; p.G63C], damaging according to the prediction tools POLYPHEN 2 and SYFT, was observed twice but the two families originate from the same area (Tuscany). Therefore, the mutation we report is the second one (different from the common ones) that is present twice in the Italian population, in 2 out of 184 pathogenic alleles.

Costa et al.[5], described the only case reported so far of a large deletion, paternally inherited, removing exon 3; they hypothesized that the deletion derived from an excision event mediated by the AluSx elements which are present in introns 2 and 3. The authors extensively discussed the mechanism of origin of the deletion, underlined that there is a 80% of homology between intron 2 and 3 of the gene, that breakpoints are within AluSx elements, close to a 21 bp tract of completely homologous sequence. They conclude that the likely mechanism is "a mispairing of homologous sequences and unequal crossing over between introns 2 and 3 of *SBDS* gene".

The deletion observed in our cases overlaps, but not completely, with the one reported by Costa et al. [5]. We aligned (BLAST sequence analysis tool) full sequences of intron 2 and intron 3 of *SBDS* and *SBDSP1* and the sequences of the 21 bp tracts present in both intron 2 and 3.

We found a high level of homology between intron 2 and intron 3 in *SBDS* and *SBDSP1* (95% for intron 2, and 94% for intron 3), while the level of homology for the three 21 bp tracts present in both intron 2 and 3 between gene and pseudogene ranged from 95,2% to 98,4%.

Carvalho et al.[6] reported an uncommon SDS genotype in a white/Hispanic female: a rare mutation [c.98A > C; p.K33T] was associated to a large insertion of an unknown segment near 5' of intron 1 or 2 in the *SBDS* gene.

When discussing the origin of the insertion, they suggested that it arose from non-homologous allelic recombination (NAHR) between *SBDS* and the closely located *SBDSP1* pseudogene.

This statement was based on the evidence that the *SBDSP*1 shares 97% identity with *SBDS* and is in an inverted orientation in a closely duplicated genomic segment. Such a structure may result in genomic instability and enhance the chance of NAHR and inversion structural variation (SV). Significantly, *SBDS* is one of the 31 disease-associated genes identified intersecting in a genome-wide analysis the location of the inverted repeats with information for gene localisation on chromosomes[7]. Other examples of diseases in which these SV are common include Hunter syndrome (IDS gene and its telomeric pseudogene IDSP1) and hemophilia A (factor VIII gene).

These data are in keeping with the high frequency of gene conversion events in generating the common pathogenic alleles found in SDS patients, and in general, the possibility of NAHR associated to the presence inverted paralogous-low copy repeats (IP-LCRs), should be considered when, for the previously quoted diseases, routine molecular testing does not immediately identify a surely pathogenic mutation.

Thus in the two unrelated families we report, this mechanism is the most likely to generate the deletion of exon 3.

Kuijpers et al.[8] and Donadieu et al [9] report failure to identify pathogenic sequence variations respectively in 5/20 and 2/104 case, whose clinical picture seemed well consistent with the diagnosis of SDS.

Therefore, it is now our policy to perform Western blotting analysis and a search for genomic rearrangements in all cases in whom a reliable clinical diagnosis is not followed by evidence of biallelic mutations.

ACKNOWLEDGEMENTS

This work was supported by grants from AISS (Associazione Italiana Sindrome di Shwachman) and from CARIPLO 2012-0529. The authors have no competing interests.

A. Minelli and L. Nacci performed the research; R. Valli analysed the data; A. Minelli and C. Danesino designed the research study and wrote the paper; G. Pietrocola directed the biochemical

analysis; U. Ramenghi, F .Locatelli, L. Brescia, E. Nicolis, M. Cipolli all contributed to collect the data.

REFERENCES

1 Dror Y1, Donadieu J, Koglmeier J, Dodge J, Toiviainen-Salo S, Makitie O, Kerr E, Zeidler C, Shimamura A, Shah N, Cipolli M, Kuijpers T, Durie P, Rommens J, Siderius L, Liu JM. Draft consensus guidelines for diagnosis and treatment of Shwachman-Diamond syndrome. Ann N Y Acad Sci. 2011 Dec;1242:40-55.

2 Boocock GR, Morrison JA, Popovic M, Richards N, Ellis L, Durie PR, Rommens JM. Mutations in SBDS are associated with Shwachman-Diamond syndrome. Nat Genet. 2003 Jan;33(1):97-101.

3 Myers K. Shwachman-Diamond Syndrome. 2008 Jul 17 [Updated 2014 Sep 11]. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews® [Internet].Seattle (WA): University of Washington, Seattle; 1993-2016.

4 den Dunnen JT and Antonarakis SE Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. Hum.Mutat. 2000;15(1):7-12.

5 Costa E, Duque F, Oliveira J, Garcia P, Gonçalves I, Diogo L, Santos R. Identification of a novel AluSx-mediated deletion of exon 3 in the SBDS gene in a patient with Shwachman-Diamond syndrome. Blood Cells Mol Dis. 2007 Jul-Aug;39(1):96-101.

6 Carvalho CM, Zuccherato LW, Williams CL, Neill NJ, Murdock DR, Bainbridge M, Jhangiani SN, Muzny DM, Gibbs RA, Ip W, Guillerman RP, Lupski JR, Bertuch AA. Structural variation and missense mutation in SBDS associated with Shwachman-Diamond syndrome. BMC Med Genet. 2014 Jun 4;15:64. doi: 10.1186/1471-2350-15-64.

7 Dittwald P, Gambin T, Gonzaga-Jauregui C, Carvalho CM, Lupski JR, Stankiewicz P, Gambin A. Inverted low-copy repeats and genome instability–a genome-wide analysis. Hum Mutat 2013; 34(1):210–220. DOI: 10.1002/humu.22217

8 Kuijpers TW1, Alders M, Tool AT, Mellink C, Roos D, Hennekam RC. Hematologic abnormalities in Shwachman Diamond syndrome: lack of genotype-phenotype relationship. Blood. 2005 Jul 1;106(1):356-61.

9 Donadieu J1, Fenneteau O, Beaupain B, Beaufils S, Bellanger F, Mahlaoui N, Lambilliotte A, Aladjidi N, Bertrand Y, Mialou V, Perot C, Michel G, Fouyssac F, Paillard C, Gandemer V, Boutard P, Schmitz J, Morali A, Leblanc T, Bellanné-Chantelot C, Associated investigators of the French Severe Chronic Neutropenia Registry. Classification of and risk factors for hematologic complications in a French national cohort of 102 patients with Shwachman-Diamond syndrome. Haematologica 2012 Sep;97(9):1312-9.